

Inventors: Lockridge and Watkins
Serial No.: 09/748,739
Filed: December 26, 2000
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ay
consists of 13 base-pair repeats separated by an 8 base-pair
spacer: 5'-GAAGTTCCTATTC[TCTAGAAA]GTATAGGAACTTC-3' (SEQ ID NO:
24). Briefly, variant libraries corresponding to the region of
butyrylcholinesterase corresponding to amino acids 277-289 (SEQ
ID NO: 13) of butyrylcholinesterase (shown as region 5 in Table
2) were transfected into mammalian cells using flp recombinase
and the 293T cell line. Table 5 shows the butyrylcholinesterase
variants S285G, P285Q and P285S that were identified and
characterized using the methods described herein utilizing Flp
recombinase and the 293T human cell line.

REMARKS

The specification has been amended in four places to
insert sequence identifiers. Concurrently with this amendment,
Applicants are filing a paper copy and computer readable form of
the sequence listing along with the appropriate statement under
Rule 821 subsections (g) and (f).

Applicants have set forth above the amendment to the
specification in clean form as required under 37 C.F.R. §
1.121(b)(1)(i) and (ii). Applicants also attach Appendix A with
marked up amendments indicated with brackets and underlining as
required under 37 C.F.R. § 1.121(b)(1)(iii). Entry of the above-
proposed amendments to the specification is respectfully
requested.

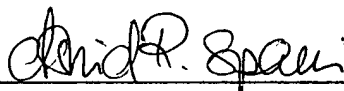
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CONCLUSION

In light of the Amendments and Remarks herein,
Applicants submit that the claims are now in condition for
allowance and respectfully request a notice to this effect.
Should the Examiner have any questions, he is invited to call
Cathryn Campbell or the undersigned attorney.

Respectfully submitted,

May 29, 2002
Date


Astrid R. Spain
Registration No. 47,956
Telephone: (858) 535-9001
Facsimile: (858) 535-8949

CAMPBELL & FLORES LLP
4370 La Jolla Village Drive
7th Floor
San Diego, California 92122
USPTO CUSTOMER NO. 23601

Attachments: Appendix A - marked up version of amendments

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APPENDIX A

At page 25, Table 1:

Human BchE (SEQ ID NO: 25)	act cct ttg tca gta
S287G (SEQ ID NO: 26)	act cct ttg ggt gta
P285Q (SEQ ID NO: 27)	act cag ttg tca gta
P285S (SEQ ID NO: 28)	act tcg ttg tca gta

The paragraph spanning pages 43 and 44:

One approach for targeting variant or heterologous nucleic acids to a single site in the genome uses Cre recombinase to target insertion of exogenous DNA into the eukaryotic genome at a site containing a site specific recombination sequence (Sauer and Henderson, Proc. Natl. Acad. Sci. USA, 85:5166-5170 (1988); Fukushima and Sauer, Proc. Natl. Acad. Sci. U.S.A. 89:7905-7909 (1992); Bethke and Sauer, Nuc. Acids Res., 25:2828-2834 (1997)). In addition to Cre recombinase, Flp recombinase can also be used to target insertion of exogenous DNA into a particular site in the genome (Dymecki, Proc. Natl. Acad. Sci. U.S.A. 93:6191-6196 (1996)). The target site for Flp recombinase consists of 13 base-pair repeats separated by an 8 base-pair spacer: 5'-GAAGTTCCTATTC[TCTAGAAA]GTATAGGAAGCTTC-3' (SEQ ID NO: 24). As described herein, the butyrylcholinesterases designated SEQ ID NOS: 4, 6, and 8, were obtained by transfection of variant libraries corresponding to region 5 of human butyrylcholinesterase (see, Table 2) into mammalian cells using Flp recombinase and the human 293T cell line. It is understood

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that any combination of site-specific recombinase and corresponding recombination site can be used in methods of the invention to target a nucleic acid to a particular site in the genome.

At pages 58 and 59, please amend the paragraph spanning pages 58 and 59 to read as follows:

Thirty-four variants were prepared using PCR-site directed mutagenesis of human butyrylcholinesterase DNA performed utilizing Pfu polymerase (Stratagene, La Jolla, CA). Three oligonucleotide primers were used to perform the mutagenesis. The mutagenesis primers were used at the same time as a general primer such as the SP6 promoter sequencing primer (MBI Fermentas, Amherst, NY) to amplify one end of the butyrylcholinesterase cDNA. The following primers were used to prepare the A328W mutant: A328W antisense 5' ATAGACTAAAAACCATGTCCCTTCATC 3' (SEQ ID NO: 29); T7 old sense 5' TAATACGACTCACTATAGGG 3' (SEQ ID NO: 30); and SP6 antisense 5' ATTTAGGTGACACTATAG 3' (SEQ ID NO: 31). The A328W primer spans 27 nucleotides and contains the A328W mutation in the middle of the primer. The PCR reaction products (megaprimers) were cleaned on QuiaQuick PCR (Qiagen, Santa Clarita, CA) according to the manufacturer's protocol to remove excess primers. The cleaned megaprimers were extended in a second PCR reaction to generate the complete 1.8 kb coding sequence of each of the 34 variants.

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At page 77, first paragraph:

As shown in Table 5, several cell lines as well as other transfection methods were also characterized. As disclosed herein, Flp recombinase also can be used to target insertion of exogenous DNA into a particular site in the genome as described by Dymecki, supra, 1996. The target site for Flp recombinase consists of 13 base-pair repeats separated by an 8 base-pair spacer: 5'-GAAGTTCCTATTC[TCTAGAAA]GTATAGGAAGCTTC-3' (SEQ ID NO: 24). Briefly, variant libraries corresponding to the region of butyrylcholinesterase corresponding to amino acids 277-289 (SEQ ID NO: 13) of butyrylcholinesterase (shown as region 5 in Table 2) were transfected into mammalian cells using flp recombinase and the 293T cell line. Table 5 shows the butyrylcholinesterase variants S285G, P285Q and P285S that were identified and characterized using the methods described herein utilizing Flp recombinase and the 293T human cell line.